H-Dbo-AAKAAW)-OH, (Dbo: 2,3-diazabicyclo[2.2.2]oct-2-ene-labeled asparagine). This distance is indicative of a rather compact peptide sampling many different coil structures, including a high PPII content, as well as turn structures. The charge lysine residue results in more turn structures being sampled by the succeeding alanine residue. UV circular dichroism (UV-CD) spectra of H-(AAKAAW)-OH and H-(AAAAAW)-OH indicate a higher PPII content for the latter peptide. These data show that the incorporation of lysine yields indeed a more compact conformation.

168-Pos

A Comprehensive Approach To Protein Thermal Stabilization

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Engineering proteins for higher thermal stability is an important and difficult challenge. We describe a comprehensive and multidisciplinary approach incorporating various individual methods to redesign proteins to be more stable. First, we identified mutations for thermal stabilization of our model, adenylate kinase by applying a variety of experimental and computational techniques individually and separately. The used techniques include X-ray crystallography, molecular dynamics simulation, domain-swapping and structural bioinformatics. Then, we designed variants by combining the individual stabilizing mutations together. The resulting variants have mutations for extra electrostatic interactions by newly added ion pairs, additional hydrophobic interaction and/or optimized local structural entropy. In the experiment using differential scanning calorimetry, the redesigned proteins displayed considerable increases in their thermal stabilities. Our results highlight the importance of a comprehensive approach in protein thermal stabilization.

169-Pos

Triple-Helix Folding Around Interruptions in the Collagen Repeating Sequence

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The collagen triple-helix consists of three supercoiled chains, each with a polyproline II-like conformation. This structure requires Gly at every third residue, and fibrillar collagens are composed of perfect $(Gly\text{-}Xaa\text{-}Yaa)_n$ repeats throughout their length. Non-fibrillar collagens, such as network-forming type IV collagen, contain interruptions in the repeated sequence that range in length from 0 to 60 residues. Folding of all collagens begins with trimerization of a terminal globular domain and is followed by propagation of the triple helix in a zipper-like manner. The effect of interruptions on this folding process is not well understood, but some delay is expected since type IV collagen, with more than 20 interruptions, folds more slowly than type I collagen, which contains no interruptions. The bacterial collagen Scl2 from *Streptococcus pyogenes* consists of a globular trimerization domain and a triple helix domain with 79 Gly-Xaa-Yaa repeats, and is an ideal system for investigating the effect of interruptions on triple helix formation. Scl2 can be expressed in *Escherichia coli*, allowing for insertion of interruptions with various lengths and sequences. Currently, we have expressed a control construct containing two collagen-like domains in tandem, and a construct with a 4-residue interruption, Ala-Ala-Val-Met, between the two collagen-like domains. The kinetics of triple-helix folding, as monitored by temperature jump experiments using circular dichroism spectroscopy and trypsin digestion/SDS-PAGE, show similar third order reaction rates for both the control protein and the protein containing the 4-residue interruption. Constructs containing larger interruptions are being expressed for future experiments. If folding is stalled at such sites, the process of renucleation around the interruptions will be characterized. In addition, fluorescence anisotropy will be used to study kinetics on a local scale and rotary shadowing electron microscopy will be used to examine single molecules.

170-Pos

Contributions of Aromatic Residues to the Folding and Stability of Human γd-Crystallin

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Human γD-crystallin (HγD-Crys), one of the most abundant proteins in the eye lens, exhibits two crystallin domains, each containing two Greek key motifs. HγD-Crys must remain folded and soluble throughout the human lifetime. Aggregation of crystallins leads to cataract.
14 tyrosines and 6 phenylalanines reside in the 173-amino-acids HγD-Crys. 16 out of these 20 residues have aromatic partners within ~4 Å. ''Tyrosine corner'' is a structural element that bridges β-strands by hydrogen bonding the tyrosine hydroxyl group and a backbone carboxyl group. Also found are interacting tyrosine/phenylalanine pairs located at the β-hairpins. These aromatic structural elements may be important in the stability and/or the folding pathways.
Site-specific mutants of the all the tyrosines and phenylalanines to alanines were constructed. Equilibrium and kinetic experiments were performed to assess stability and unfolding/refolding rates.
For stability, all the N-terminal domain (N-td) mutants had the N-td destabilized, but C-td unaffected, with increased population of the single-domain-folded intermediate, although the extents of destabilization were different. All the C-td mutants had both the N-td and C-td destabilized, showing a more cooperative folding process that was best fit to a two-state model, and similarly, the degrees of destabilization varied. Selected tyrosines were mutated to phenylalanines with very little effect on the N-td or the C-td stabilities. For kinetics, C-td mutants had accelerated unfolding rates, while N-td mutants had no effect. Y45A, Y50A Y133A, Y138A had slower refolding rates, while other mutants had no effects.
The results were consistent with a sequential unfolding pathway, in which the N-td of HγD-Crys unfolds first, followed by the C-td. The aromatic residues were almost all important in the mature stability of HγD-Crys, while a subset of these aromatic residues were important determinants of unfolding/refolding rate.

171-Pos

Role of Proline in the Folding of Conotoxins

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Evaluation of the role of disulfide bridges plays an important part in understanding the concept of protein folding. We are investigating how slight changes - the presence *vs.* the absence of the cyclic amino acid proline in a certain position of the peptide-chain - in the sequence of small peptides influence their folding properties. The present studies focus on the folding of a group of small peptides found in *Conus* snails, α-conotoxins SI, SIA (found in *Conus Striatus*), and GI (*Conus Geographus*), under two different oxidizing conditions. Each peptide has two disulfide bridges leading to three possible regioisomers, only one of which is found in nature. Our results indicate that peptides containing the cyclic amino acid proline had very high selectivity for the natural isomer, suggesting that this amino acid enforces a structural rigidity on the peptides.
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172-Pos

Investigation of W121 on the Conformation and Functional Properties of the Human Acidic Fibroblast Growth Factor-1

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Fibroblast Growth Factor-1 (FGF-1) is a 16kDa heparin binding protein, which has been associated with a variety of important functions including angiogenesis and wound repair. In order for FGF-1 to enter the cell it must interact with the FGF-1 receptor on the cell surface. One vital residue involved in the binding of FGF-1 to the receptor is tryptophan 121 (W121). This study aims to examine the role of W121 on the conformation and functionality of FGF-1. Site-directed mutagenesis will be used to incorporate mutations at position 121. The effect of these mutations will be characterized using various biophysical techniques including fluorescence, CD, ITC, and mutli-dimesional NMR spectroscopy. As FGFs are involved in many crucial cellular processes, the gain from this study is expected to provide useful information on the regulation of the FGF signaling process.

173-Pos

Characterization of the Minimalistic Fgf-D2 Domain Interface

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Fibroblast growth factors (FGFs) are heparin binding proteins that help regulate key cellular processes such as wound healing and differentiation, cell proliferation, cell migration, morphogenesis, and angiogenesis. FGF signaling is generated by the binding of the ligand (FGF) to the extracellular domain of the FGFR; heparin is believed to play a major role in this interaction. The role of heparin on the FGF-D2 complex was determined by using sucrose octasulfate, an analogue of heparin, role in the FGF-D2 complex. In this study we have determined the three dimensional structure of the minimalistic FGF-D2 domain interface using a variety of biophysical techniques including multidimensional NMR spectroscopy. The SOS and FGF binding sites on D2 have been mapped using 1H-15N HSQC. The three dimensional structure of the minimalistic FGF signaling complex, has been validated by site-directed mutagenesis studies. The results of this study provide valuable information towards a rational design of therapeutic principles against FGF-induced pathogenesis.